

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
14 December 2000 (14.12.2000)

PCT

(10) International Publication Number
WO 00/74689 A1

(51) International Patent Classification⁷: **A61K 31/715, 35/78**

(74) Agents: REED, Janet, E. et al.; Saul, Ewing, Remick & Saul LLP, Centre Square West, 1500 Market Street, 38th Floor, Philadelphia, PA 19102-2186 (US).

(21) International Application Number: **PCT/US00/15693**

(81) Designated States (*national*): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(22) International Filing Date: 8 June 2000 (08.06.2000)

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(25) Filing Language: English

Published:

— *With international search report.*

(26) Publication Language: English

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(30) Priority Data:
09/328,611 9 June 1999 (09.06.1999) US

(71) Applicant (*for all designated States except US*): WISCONSIN ALUMNI RESEARCH FOUNDATION [—/US]; 614 Walnut Street, Madison, WI 53705 (US).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): MARLETT, Judith, A. [US/US]; 653 Odell Street, Madison, WI 53711 (US). FISCHER, Milton, H. [US/US]; 6624 Wood Circle East, Middleton, WI 53562 (US).

WO 00/74689 A1

(54) Title: UNFERMENTED GEL FRACTION FROM PSYLLIUM SEED HUSKS

(57) Abstract: A purified form of a gel-forming component of psyllium seed husks is disclosed, along with a process for obtaining the gel-forming fraction from psyllium seed husks. Methods of using this gel-forming fraction as a laxative and hypocholesterolemic agent are also disclosed.

**UNFERMENTED GEL FRACTION FROM
PSYLLIUM SEED HUSKS**

Pursuant to 35 U.S.C. §202(c), it is acknowledged that the U.S. Government has certain rights in the invention described herein, which was made in part with funds from the National Institutes of Health, Grant 5 No. DK21712.

This application claims priority to U.S. Application No. 09/328,611, filed June 9, 1999, the entirety of which is incorporated by reference herein.

10 **FIELD OF THE INVENTION**

This invention relates to the field of laxatives, treatments to lower blood serum cholesterol and low-calorie food thickeners and fat substitutes. In particular, the invention relates to unfermented gel-forming polysaccharides from psyllium seed husks and methods for their isolation. 15

BACKGROUND OF THE INVENTION

Various scientific and scholarly articles are 20 referred to in parentheses throughout the specification. These articles are incorporated by reference herein to describe the state of the art to which this invention pertains.

The seed husks of psyllium (*Plantago ovata*, 25 also known as ispaghula) are commonly used as a laxative and to promote regular bowel function. Psyllium seed husks promote laxation partially by increasing the mass and moisture content of the stool (Marteau et al., 1994, Gut 35:1747-1752). Additionally, the excreta of animals 30 and humans fed diets containing psyllium seed husk is

5 gelatinous. This gelatinous property contributes to laxative properties of psyllium seed husks by decreasing friction in the gut. Observed increases in fecal mass and water retention also have been attributed to this
5 gelatinous material (Marteau et al., 1994, *supra*). The gel is composed largely of unfermented psyllium polysaccharides (Cabotaje et al., 1994, 1302-1307).

10 Currently used preparations of psyllium seed husks have certain disadvantages. Laxative preparations of psyllium seed husks are generally composed of ground husk and have coarse and unpleasant mouthfeel when administered in drinks. Psyllium seed husks have been incorporated into cookies, crackers and similar products; however, these products have a tendency to begin to gel
15 unpleasantly in the mouth. More significantly, though, psyllium seed husks can swell in the esophagus, producing an esophageal obstruction that can cause choking. For this reason, psyllium seed husk preparations are not recommended for ingestion by persons who may have
20 difficulty swallowing (e.g., elderly persons). Finally, the recommended daily dose of psyllium husk of 3.5 - 11 g per day is inconvenient to ingest in any form. What is needed is a form of psyllium husk that is convenient and pleasant to use.

25 Psyllium seed husk has many of the properties of soluble dietary fiber sources. Commonly used sources of soluble dietary fiber include pectin, gums and oat bran. Soluble dietary fiber (SDF) has many uses in food and medicinal preparations. Soluble fibers are
30 components of minimally processed food sources such as oats, oat bran and barley, or are available as concentrates, such as gums, pectins and mucilages. Gums and mucilages are carbohydrate polymers that are generally isolated from plant sources. Mucilages in
35 particular produce slippery or gelatinous solutions in

water. Pectins are polymeric chains of partially methylated galacturonic acids that also possess the ability to form a gel in water. Most soluble fibers are rapidly and completely fermented and have no laxation properties.

Sources of soluble dietary fiber that are also viscous lower serum cholesterol in animals and humans (Marlett, 1997, pp. 109-121, Dietary Fiber and Health, Plenum Press, New York, ed. Kritchevsky and Bonfield). The viscosity of the SDF, rather than its fermentation in the gastrointestinal tract, is key to its hypcholesterolemic action (Marlett et al., 1994, *Hepatology* 20:1450-1457). Viscosity in the lumen of the lower small intestine interferes with the absorption of bile acids and more bile acids are lost through the stool. Blood cholesterol is thought to be lowered primarily because it is being used in the liver to synthesize more bile acids to replace those lost. The synthesis of bile acids in the liver accounts for 40 to 50% of the daily elimination of cholesterol from the blood. However, the addition of one source of soluble fiber, oat bran, to the diet also increases the proportion of deoxycholic acid in the bile acid pool, which decreases the absorption of exogenous dietary cholesterol. Supplementing the diet with psyllium husk also increases the excretion of bile acids by about 50% (Gelissen et al., 1994, *Am. J. Clin. Nutr.* 59:395-400).

Soluble dietary fiber concentrates are also often used as thickeners and low calorie fat substitutes in the food industry because of their hydrocolloidal properties (Ward, 1997, *Cereal Foods World*, 42:386-390). Low-viscosity gums such as gum acacia have both hydrophilic and lipophilic properties that make them ideal as emulsifiers, surfactants and stabilizers. Pectins and mucilages have gel-forming properties that

made them ideal thickeners of food products. Pectins are traditionally extracted from apple and citrus fruits. Commonly used mucilages are generally extracted from seaweed and include carrageenan, agar and alginate. A 5 fat substitute can be made by combining gum with mucilage and/or pectin to create a compound with the emulsifying properties and smoothness of a fat.

SUMMARY OF THE INVENTION

10 The present invention provides the gel-forming component of psyllium seed husks in a purified form. This gel fraction provides the laxation and hypcholesterolemic effects of intact psyllium seed husks, but is in a form that is easily administrable as a 15 tablet, capsule or liquid, without certain unpleasant or unsafe qualities associated with the use of intact psyllium seed husks. The gel fraction also has utility in treatment of other intestinal abnormalities and maintaining normal bowel function, and as a food 20 thickener and fat replacement.

According to one aspect of the invention, a gel-forming fraction of psyllium seed husks that survives microbial fermentation upon passage through a monogastric mammalian digestive tract is provided. Among other 25 components, the gel fraction comprises predominantly xylose and arabinose in a dry weight ratio of at least about (preferably about 3.5). The fraction comprises notably limited amounts of other sugars, e.g., about 2.5% - 13.5% total of rhamnose, galactose, glucose and uronic 30 acids. More specifically, the gel-forming fraction has the following sugar composition, expressed as a percentage of total sugars:

35 between about 0.5% and 4% rhamnose;
between about 19% and 22% arabinose;
between about 68% and 76% xylose;

between about 0% and 0.5% mannose;
between about 1% and 2% galactose
between about 0% and 1% glucose; and
between about 1% and 6% uronic acids

5 Upon further purification, the gel-forming fraction becomes even more depleted in rhamnose, glucose and uronic acids.

The gel-forming fraction is also highly viscous, a 0.2% concentration in formamide having an 10 apparent viscosity of at least 500 sec, preferably 750 sec, and most preferably 850 sec. The fraction is soluble in a dilute alkaline solution and forms a gel upon acidification of the solution to a final pH of about 4.5.

15 According to another aspect of the invention, in a preferred method of obtaining the psyllium seed husk gel-forming fraction, a separate carbohydrate fraction is also obtained. This fraction is soluble in the dilute alkaline solution and remains soluble upon acidification 20 of the solution to a pH of about 4.5. This fraction is comprised of xylose and arabinose in a ratio of at least about 4:1 and further comprises at least about 12% (by weight) rhamnose and at least about 15% (by weight) uronic acids.

25 According to another aspect of the invention, a method of fractionating psyllium seed husks to obtain a gel-forming fraction and an additional carbohydrate fraction is provided. The method comprises: (a) mixing the husks, in the presence of a chemical reducing agent, 30 in an aqueous alkaline solution comprising between about 0.15 and about 1.0 M (preferably 0.15 - 0.5 M, more preferably 0.15 - 0.4 M, even more preferably 0.15-0.3 M and most preferably 0.15-0.2 M) hydroxyl ions, thereby fractionating the husks into an alkali soluble fraction 35 and an alkali-insoluble fraction; (b) removing the alkali

insoluble fraction; (c) acidifying the alkali soluble fraction to a pH of between about 3 and about 6 (preferably between about 4 and about 5, most preferably about 4.5), which results in the gelation of the gel-forming fraction; and (d) separating the gel fraction, e.g., by centrifugation or other means, from the additional carbohydrate fraction contained in the acidified solution. In preferred embodiments, the method further comprises washing the gel fraction with an aqueous or buffered solution and desiccating the washed gel fraction.

In another aspect of the invention, a gel-forming fraction from psyllium seed husks is provided, which is produced by the aforementioned procedure. The additional carbohydrate fraction is also provided in this aspect of the invention.

According to another aspect of the invention, alternative methods are provided for obtaining a polysaccharide fraction that contains the aforementioned gel-forming fraction. Such a fraction is obtained by solvent extraction using formamide, dimethylsulfoxide or 4-methylmorpholine N-oxide (50% solution in water). The solvent-treated material is centrifuged to recover the soluble materials that are then poured into ethanol to achieve a concentration of 80% ethanol. The precipitate that forms is similar in composition to the alkali-soluble gel-forming fraction.

According to another aspect of the invention, pharmaceutical preparations for treatment of constipation or other intestinal abnormalities, or for lowering serum cholesterol levels in a patient are provided. These preparations are formulated to contain effective dosages of the psyllium seed husk gel-forming fraction of the invention. Methods of treating patients for these various conditions are also provided, which comprise

administering the pharmaceutical preparations of the invention.

Other features and advantages of the present invention will become apparent from the detailed 5 description and examples that follow.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a highly polymerized gel-forming fraction from psyllium seed husks 10 that has great utility in the treatment and prevention of constipation and as a hypcholesterolemic agent. This gel fraction remains substantially unfermented during transit through the gastrointestinal tract (Example 6 and Example 7) and promotes laxation through a variety of 15 means, including increasing moisture content and overall mass of stool and imparting to the stool a slippery characteristic that facilitates ease of passage of stool (Example 6).

The gel-forming fraction from psyllium seed 20 husks has also been demonstrated as a hypcholesterolemic agent. Accordingly, the gel-forming fraction may be used alone or in combination with other active substances as a therapeutic treatment to lower serum cholesterol.

Stool from rats and humans fed psyllium seed 25 husks are sometimes gelatinous (Cabotaje et al., 1994, *supra*; Example 6). However, attempts to purify and characterize a gel-forming material (Kennedy et al., Carbohydrate Res. 75:265-274, 1979; Sandhu et al., Carbohydrate Res. 93:247-259, 1981) have not been 30 successful and none has provided the highly purified gel-forming fraction having the features described in accordance with the present invention. The method of fractionating psyllium seed husks developed in accordance with the invention has led to the unexpected discovery, 35 contrary to published reports (e.g., Kennedy et al.,

1979, *supra*), that a gelatinous, alkali-soluble fraction of psyllium seed husks can be further fractionated to form a highly viscous gel fraction (referred to herein as "Fraction B") and a second carbohydrate fraction with 5 distinctive compositional features, as described in greater detail below (referred to herein as "Fraction C"). Both the viscous, gel-forming Fraction B and the additional Fraction C are soluble in a variety of substances, including dilute and concentrated alkali, 10 formamide, dimethylsulfoxide and 4-methylmorpholine N-oxide (50% aqueous solution); consequently, these two fractions together can be isolated on the basis of these solubility characteristics. However, the further separation of the fractions has been either unsuccessful 15 (e.g., a strong alkali-extracted gel-forming fraction was unable to be further separated by Kennedy et al., 1979; *supra*) or has remained unexplored. The present inventors have discovered that, using a suitable first extraction procedure to obtain a product comprising fractions B and 20 C together, that the fractions can be separated by acidification of a solution containing a mixture of the fractions. Fraction B is concentrated for separation from the acidified mixture by centrifugation, whereas Fraction C remains soluble in the acid.

25

Description of Carbohydrate Polymers

The viscous, gel-forming psyllium seed husk fraction of the invention (Fraction B) is comprised primarily of xylose and arabinose. In a preferred 30 embodiment, the gel-forming fraction has at least 50% xylose and arabinose by weight, in a more preferred embodiment at least 75% xylose and arabinose by weight, and in a most preferred embodiment at least 85% xylose and arabinose by weight. The gel-forming fraction has an 35 apparent viscosity, as determined in the method of

Example 3, of at least 500 sec, in a more preferred embodiment of at least 750 sec, and in a most preferred embodiment of at least 850 sec. The gel-forming fraction is furthermore particularly deficient in rhamnose,

5 galactose and uronic acids, as compared to xylose. In a preferred embodiment, the ratio of weights of xylose to rhamnose is more than 50, in a more preferred embodiment the ratio is more than 60, and in a most preferred embodiment, the ratio is more than 65. In a preferred embodiment, the ratio of weights of xylose to galactose

10 is more than 25, in a more preferred embodiment the ratio is more than 35, and in a most preferred embodiment, the ratio is more than 42. In a preferred embodiment, the ratio of weights of xylose to uronic acid is more than

15 15, in a more preferred embodiment the ratio is more than 25, and in a most preferred embodiment, the ratio is more than 35. In a preferred embodiment, the ratio of weights of xylose to arabinose of Fraction B is between 2.5 and 4.5, in a more preferred embodiment the ratio is between

20 3.0 and 4.0, and in a most preferred embodiment, the ratio is between 3.25 and 3.75.

The acid-soluble psyllium seed husk fraction (Fraction C) is also high in xylose and arabinose. In a preferred embodiment, the acid-soluble fraction has at least 25% xylose and arabinose by weight, in a more preferred embodiment at least 40% xylose and arabinose by weight, and in a most preferred embodiment at least 45% xylose and arabinose by weight. Though Fraction C has an apparent viscosity similar to that of fraction B, it does not have the gel-forming property of Fraction B.

Fraction C is furthermore particularly enriched in rhamnose, galactose and uronic acids, as compared to xylose. In a preferred embodiment, the ratio of weights of xylose to rhamnose is less than 6.0, in a more preferred embodiment the ratio is less than 4.5, and in a

- 10 -

most preferred embodiment, the ratio is less than 3.0. In a preferred embodiment, the ratio of weights of xylose to galactose is less than 40, in a more preferred embodiment the ratio is less than 30, and in a most preferred embodiment, the ratio is less than 25. In a preferred embodiment, the ratio of weights of xylose to uronic acid is less than 30, in a more preferred embodiment the ratio is less than 10, and in a most preferred embodiment, the ratio is less than 5.0. In a preferred embodiment, the ratio of weights of xylose to arabinose of Fraction C is more than 3.0, in a more preferred embodiment the ratio is more than 4.0, and in a most preferred embodiment, the ratio is more than 4.5.

15 **Preparation of Psyllium Seed Husk Fractions**

The present invention also provides methods of fractionating psyllium seed husks to yield the purified and separated fractions described above. In its most basic form, the method has the following steps:

20 1. Suspend psyllium seed husks in a dilute alkaline aqueous solution (preferably 0.15 - 0.2 M, hydroxyl ions) containing a reducing agent, in which portions of the husk material will dissolve, while a certain portion remains insoluble;

25 2. Remove the alkaline-insoluble material (referred to herein as "Fraction A"), e.g., by centrifugation;

30 3. Acidify the alkali-soluble fraction of step one to a pH of between 3 and 6, preferably 4.5, to yield a gel (Fraction B) and an acid-soluble fraction (Fraction C); and

35 4. Separate the gel from the acidified solution, e.g., by centrifugation.

One example of this method is taught in Example 35 1. Many variations exist to the method that will not

- 11 -

substantially change the product isolated. These are described in detail below.

The alkaline solubilization step has several variations. The method taught in Example 1 has improved 5 this solubilization over that found in the prior art. Previous alkaline solubilizations of psyllium seed husk polysaccharides utilized concentrated solutions of base (i.e. 1.2 M NaOH, Kennedy et al., 1979, *supra*) without a reducing agent. Recognizing the harsh nature of this 10 treatment and its partial degradation of polysaccharide chains in the gel-forming fraction, the inventors have demonstrated that a gel-forming fraction can be obtained, presumably in a form more suitable for further fractionation, using a much less concentrated alkaline 15 solution and a suitable chemical reducing agent, such as borohydride. Though up to 4 N alkaline solution can be utilized, the concentration of base in the alkaline solubilization is preferably at least 0.15 N and not more than 1.0 N; in a more preferred embodiment, at least 0.15 20 N and not more than 0.5 N; and in the most preferred embodiment, at least 0.15 N and not more than 0.2 - 0.3 N. Any standard base can be used in the alkaline extraction, including, but not limited to, sodium 25 hydroxide, potassium hydroxide, lithium hydroxide, ammonium hydroxide and tetramethyl ammonium hydroxide.

A chemical reducing agent, such as borohydride, should be added to the alkaline solubilization step to minimize base-catalyzed depolymerization. In Example 1, a concentration of 1 g/L of sodium borohydride is used, 30 but effective concentrations range from about 50 mg/L to 10 g/L. In a preferred embodiment, the sodium borohydride concentration is at least 100 mg/L and not more than 4 g/L, in a more preferred embodiment at least 500 mg/L and not more than 2 g/L, and in a most preferred embodiment, 35 at least 800 mg/L and not more than 1.2 g/L. Other forms

of borohydride also are suitable for use in this step, including but not limited to, lithium borohydride, potassium borohydride and sodium cyanoborohydride.

The degree of initial processing of psyllium
5 seed husks may alter the alkaline solubilization in ways
that will be well known to those skilled in the art. It
is important that the husk material be processed so that
it is in small pieces, in order to allow the viscous
polysaccharides to be easily separated from the insoluble
10 and fibrous materials of the cell walls. In Example 1,
the psyllium seed husks are milled, but any process that
pulverizes the plant material can be used, and these
processes are well known in the art.

The ratio of seed husk material to alkaline
15 solution can be important for efficient solubilization of
the polysaccharide fractions. In Example 1, a ratio of 2
g psyllium seed husk is added to 400 ml of alkaline
solution, but this ratio can be varied without
appreciably affecting the solubilization. For instance,
20 the ratio can be varied so as to add as little as about
0.1 g seed husks or as much as about 4 g seed husks to a
400 ml alkaline solution. Additionally, the time of
solubilization can be varied to optimize the procedure
(0.5 hr - 24 hr) at a range of temperatures (4-50°C).

25 Step two of the method of the invention
requires that the alkaline insoluble materials be
separated from the alkaline soluble materials. In
Example 1, centrifugation is employed to accomplish this
objective. However, numerous variations and other
30 procedures may be substituted without substantially
changing the soluble materials isolated. One skilled in
the art will know how to alter the time and force of the
centrifugation to adapt the separation to different
centrifuge rotors, plant materials and alkaline
35 solutions. Other methods that will accomplish this

separation are well known in the art. Some of these methods will be better suited to large scale use of the method of the invention. Separation methods of interest include, but are not limited to, flow-through

5 centrifugation or filtration (with agitation). Example 1 further teaches washing the insoluble materials with the alkaline solution and re-separation to improve the yield of the alkaline soluble materials. This washing step is optional but can be used to advantage to improve yield.

10 Step three requires that the alkaline soluble materials of step two be subjected to acidification. In Example 1 this is accomplished by adding glacial acetic acid to the combined alkaline soluble materials until the pH is adjusted to 4.5. The range of pH used for this

15 acid solubilization can be varied without substantial effect on the products. In a preferred embodiment, the pH is between 3 and 6, in a more preferred embodiment, the pH is between 4 and 5, and in a most preferred embodiment, the pH is about 4.5 as described in Example

20 1. The choice of acid is also subject to variation. Examples of acids suitable for use in this step are acetic, hydrochloric, sulfuric, oxalic, trichloroacetic and trifluoroacetic acids, among others. Here as in step one, the duration, temperature, etc. of the

25 solubilization can be varied, but preferably is carried out at ambient temperature for about 2 hours.

Step four requires that the acid insoluble gel-like material (Fraction B) be separated from the acid soluble materials (Fraction C). Centrifugation is

30 typically employed to accomplish this separation. An optional washing of the insoluble gel mass (e.g., with water, buffer or other suitable solvent) may also be performed to improve the efficiency of the separation.

As alternatives to centrifugation in step four,

35 two other approaches, both of which are amenable to large

- 14 -

scale preparations, may be utilized. The inventors have observed that gel-like material of Fraction B floats, and that the gel has good integrity. Accordingly, the gel can be strained or skimmed from the acidified mixture 5 using a paddle, for instance, such as the type used to remove curds during cheese-making. The gel material is thereafter deposited into a separate container, where it may be washed as a further purification step. Alternatively, the vessel containing the acidified 10 solution with the gel floating on top can be drained from the bottom by gravity or by gentle vacuum, to leave the gel at the bottom of the vessel. Again, the gel may be washed.

Preparing Fraction B and Fraction C for storage 15 and/or use may employ several procedures. The polysaccharide preparations of Fractions B and C may be used or stored hydrated. If stored in a hydrated form, preservatives or bacteriostatic agents may be added. Drying the polysaccharide preparations is particularly 20 advantageous for use or storage. In a preferred embodiment, Fraction B and Fraction C are desiccated by treatment with 95% ethanol, washing with diethyl ether and drying. The fractions also may be desiccated with other solvents, such as methanol, acetone or isopropyl 25 alcohol. Any standard dehydration method (e.g., evaporation, lyophilization) may be used to dry the fractions, provided the temperature is maintained at less than about 60°C, more preferably at less than about 40°C.

30 **III. Uses of Psyllium Seed Husk Fractions**

The psyllium seed husk fractions of the invention have uses as therapeutic treatments. In this regard, the viscous, gel-forming fraction, Fraction B, has been demonstrated effective in promoting laxation and 35 also as a hypocholesterolemic agent. This material can

- 15 -

be used alone or in combination with other active substances in therapeutic or prophylactic preparations for constipation, diarrhea and/or high serum cholesterol. Such preparations can incorporate the gel-forming

5 fraction in pills, capsules or liquids to be administered by mouth. In a preferred embodiment, a dried form of the gel is formulated for convenient administration as a pill or capsule. The gel re-hydrates upon ingestion. With regard to re-hydration, the gel-forming fraction

10 possesses hydration characteristics that are particularly advantageous. After the gel-forming fraction has been isolated and dried, it is slow to hydrate. However, based on observations following feeding the material to colectomized rats, it is clear that the gel does become

15 hydrated in the upper gut, where it exerts its hypocholesterolemic effect. This delay in hydration is advantageous inasmuch as the risk of premature hydration, e.g., by a delay in the esophagus, is minimized or avoided.

20 The gel-forming preparations can additionally be incorporated into food products. Since the active polysaccharides have been isolated away from the other plant cell components by the method of the invention, they will not have the unpleasant mouthfeel or the

25 necessity to administer large dosages associated with the psyllium seed husk preparations currently in use.

The unfermented gel-forming polysaccharide from psyllium seed husks is well-known for its laxative effects on the monogastric mammalian digestive tract.

30 For an adult human, a suitable dosage of the gel-forming fraction in dry form is about 2 g, one to three times a day, to maintain bowel regularity and as a treatment for constipation.

As described in Example 4, the gel-forming

35 fraction of psyllium seed husks has been demonstrated as

- 16 -

a hypocholesterolemic agent. Accordingly, this fraction also may be used alone or in combination with other active substances as a therapeutic treatment to lower serum cholesterol. For an adult human, a suitable dosage 5 of Fraction B in dry form is from about 3 g to about 7 g daily. The psyllium husk Fractions B and C of the invention can additionally be used as food additives. They may be used as thickeners, gel-formers and bulking agents in prepared foods. They may also be combined with 10 other food additives to make fat mimetic systems. Because the polysaccharide preparations of the invention are partially non-digested, they will additionally be low calorie, serum cholesterol-lowering and laxation-promoting. The polysaccharide preparations of Fractions 15 B and C can be used in many of the food products where gums and mucilages are currently used.

The following examples are provided to describe the invention in greater detail. They are intended to 20 illustrate, not to limit, the invention.

25

30

35

EXAMPLE 1

5 Fractionation of Psyllium Seed Husks

Milled psyllium seed husks (2 g) were stirred with 0.2 N potassium hydroxide (400 ml) containing sodium borohydride (400 mg) in a nitrogen atmosphere for 90 10 minutes. The mixture was centrifuged for 20 minutes at 23,500 x g. The supernatant was decanted from an insoluble fraction that had settled out in the centrifuge bottle. The insoluble fraction (designated "Fraction A") was stirred with fresh potassium hydroxide-sodium 15 borohydride solution (100 ml) for an additional 15 minutes and re-centrifuged. The supernatants from both centrifugations were combined. The pH of the combined supernatants was adjusted to 4.5 with glacial acetic acid, with stirring, at ambient temperature, then 20 centrifuged for 60 minutes at 25,500 x g. The supernatant (Fraction C) was decanted from a gel mass (Fraction B) that had settled out in the centrifuge bottle. The gel was washed gently with water (50 ml) to remove adhering supernatant solution and this wash water 25 was added to the supernatant. The gel fraction was desiccated by treatment with 95% ethanol and finally washed with diethyl ether and dried.

Prior to desiccating Fraction B, in some instances the fraction was further purified by repeating 30 the alkaline solubilization and acidification steps described above. The gel was re-suspended in 0.2 N KOH, acidified to pH 4.5 and centrifuged to recover the gel.

- 18 -

EXAMPLE 2
Measurement of the Sugar Composition of
Psyllium Seed Husk Fractions B and C

5 The respective sugar compositions of the gel fraction (Fraction B) and the acid soluble fraction (Fraction C) produced by the method set forth in Example 1 were determined. Results are shown in Table 1.

10 **Table 1.** Composition of psyllium seed husk fractions (dry wt.).

	Psyllium Seed Husks (2 g)	Alkali Insoluble (Fraction A)	Alkali Soluble	
			Acid Insoluble (Fraction B)	Acid Soluble (Fraction C)
	Yield (g)	--	0.328	1.164
<hr/>				
15	Composition (% dry wt)			
	Rhamnose	3.17	0.49	0.95
	Arabinose	19.93	33.58	19.45
	Xylose	49.15	3.16	67.20
20	Mannose	2.17	10.84	0.0
	Galactose	3.83	12.86	1.59
	Glucose	4.37	19.34	0.37
	Uronic Acids	5.41	3.58	1.74
<hr/>				
25	Ratios of % dry wts			
	Xylose/ Rhamnose	15.50	6.45	70.70
	Xylose/ Arabinose	2.47	0.094	3.46
30	Xylose/ Galactose	12.83	0.25	42.26
	Xylose/ Uronic Acids	9.08	0.88	38.62
				2.30
<hr/>				

- 19 -

EXAMPLE 3

**Measurement of the Viscosity of the
Psyllium Seed Husk Gel-Forming Fraction**

5 Apparent viscosity of the gel-forming fraction
(Fraction B) of psyllium husk was determined. The
solution for apparent viscosity determination was
prepared by stirring overnight a 0.2% solution of the dry
fraction in formamide. The solution was then heated with
10 stirring to 70°C over a 5 minute period, and stirred for
1 hour at ambient temperature. The apparent viscosity
was measured using a pipet viscometer (Ostwald Dropping
Pipet Viscometer, Cat. No. 13-695, Fisher Scientific,
Pittsburgh, PA). The viscosity of the solution was
15 measured three times and mean values determined.

**Table 2. Apparent viscosity of psyllium seed husk
Fraction B.**

	Apparent Viscosity (sec)
20 Alkaline Soluble, Acid	876
Insoluble Fraction	
(Fraction B)	
Formamide (solvent only)	303

25

EXAMPLE 4

Solvent Extraction of Psyllium Seed Husk Components

30 Psyllium seed husk components may also be
obtained by extraction with various solvents. Such
solvents include formamide, dimethylsulfoxide and 4-
methylmorpholine N-oxide (50% solution in water).

35 Milled psyllium seed husk (2 g) was added in
small portions to a selected solvent (200 ml) with
stirring over 30-60 min. In one protocol, the mixture
was stirred for two days at room temperature, then

- 20 -

centrifuged for 40 min at 27,000 x g. In alternative protocols, the mixture was stirred at elevated temperatures, up to 60°C, whereupon the length of stirring could be reduced to as little as 12 hours.

5 The pelleted insoluble material was resuspended and stirred in additional solvent (50 ml) for 30 min, then re-centrifuged. The combined supernatants were added, with stirring, to 95% ethanol (5 volumes), bringing the final ethanol concentration to 80%.

10 Methanol, isopropyl alcohol, acetone or similar solvents could be substituted for ethanol in this step. The precipitate was collected and washed with absolute ethanol, followed by diethyl ether, then dried.

15 The ethanol-insoluble precipitate that forms is similar in composition to the alkali-soluble material described in Examples 1-3. The sugar compositions of fractions obtained by the foregoing initial solvent extraction are shown in Table 3.

20 Table 3. Sugar compositions of fractions (% dry wt)

	Formamide	4-Methyl-morpholine N-oxide	Dimethyl sulfoxide
Rhamnose	3.03	3.25	0.48
Arabinose	18.95	16.54	17.38
25 Xylose	65.56	57.47	65.33
Mannose	0.05	0.0	0.12
Galactose	1.54	1.50	1.64
Glucose	0.21	0.22	0.34
Uronic Acids	4.30	4.69	0.94

30

EXAMPLE 5

**Effect of Psyllium Seed Husks and Fractions
Thereof on Reabsorption of Bile Acids from
the Small Intestines of Rats**

5

One of the major sites for the hypocholesterolemic action of dietary fiber is in the lower small intestine (ileum), through its effect on bile acids. Bile acids function as emulsifiers in the small intestine to facilitate fat digestion and absorption. They are synthesized from cholesterol in the liver, stored in the gall bladder and secreted into the small intestine in response to eating. Bile acids are conserved through re-absorption in the ileum and recycling back to the liver through the blood. Food is digested and fluid absorbed as digesta moves distally in the intestine, such that in the lower third of the small bowel, the luminal contents consist largely of indigestible material (i.e. fiber) and some fluid. It is now known that many of the viscous soluble fiber sources retain their viscosity during transit through the upper gastrointestinal tract, and essentially become concentrated in the lower gut. The soluble fiber effectively interferes with the re-absorption of bile acids in the ileum, the only site in the gut that has the necessary transport proteins for bile acids. Bile acids lost through stool are replaced by new synthesis in liver from blood cholesterol, which effectively lowers blood cholesterol levels.

30

Rats were surgically modified by removing the cecum and colon, and re-connecting the end of the ileum to the rectum. After a recovery period of 7-10 days, these animals secrete soft, formed material referred to as ileal excreta. Ileal excreta is preferred to stool for measuring the effect of a substance on absorption of bile acids, because bacterial transformation of bile

- 22 -

acids in the large intestine can render up to half of them unidentifiable in stool.

Test meals were fed to groups of rats. Each meal was fed to four rats; the test meals contained 5% fiber as either (1) psyllium seed husk (PSH); (2) cellulose (as a control); or (3) the amount of a selected seed husk fraction (A, B or C), singly or combined, that would be present in 5% psyllium seed husks. The test meals also contained a non-absorbable marker so that the concentration of the marker in the ileal excreta could be used to calculate of the amount of the test meal that was present in the ileal excreta collected.

The results of the acute test meal study are shown in Table 4.

15

Table 4. Bile acids excreted after feeding test meals containing various soluble fibers.

Test Meal	Bile acids excreted* ($\mu\text{mol/g}$ test meal)
Cellulose	8.32 \pm 2.42
Unmilled PSH	18.41 \pm 2.61
Fraction A	9.07 \pm 2.75
Fraction B	16.08 \pm 3.20
Fraction C	13.76 \pm 2.71
Combined A,B,C	15.65 \pm 1.37

Mean \pm SEM, n = 4 rats

An increase in bile acids excreted indicates a concomitant decrease in bile acids re-absorbed into the blood. An agent that prevents re-absorption of bile acids into the blood from the ileum is considered to have hypocholesterolemic properties. The results above demonstrate the hypocholesterolemic properties of psyllium seed husks and, more notably, of the conveniently-administrable gel-forming fraction, Fraction B, which was essentially equivalent to intact psyllium seed husks in hypocholesterolemic effect.

EXAMPLE 6
The Unfermented Gel Component of
Psyllium Seed Husks Promotes
Laxation as a Lubricant in Humans

5

In addition to increasing stool weight, a supplement of psyllium seed husks (PSH) produces a stool that is slick and gelatinous. In this example, we demonstrate that a gel fraction of psyllium escapes microbial fermentation and is responsible for these characteristics that enhance laxation.

10

Materials and Methods:

Experimental design. The study consisted of 3 periods, a screening phase, the PSH period and the basal period. During the screening phase, subjects consumed 15 g/d of Metamucil® (5g/meal) (Smooth Texture Metamucil®, The Procter & Gamble Co., Cincinnati, OH), along with their usual diet, for 12 days to provide an additional 8.8 g/d of dietary fiber. Subjects were asked to complete food intake records during d 9-12 to evaluate compliance with a protocol and to obtain more information about typical food intakes. Two stools were obtained from each subject during d 9-12 also to evaluate compliance with the protocol. During the next 7 days (d 13-19 of the study), subjects consumed a defined, low fiber diet, and with each meal, the PSH and a nonabsorbable marker (chromic sesquioxide, 200 mg/meal). For the next two weeks (d 20-33 of the study), subjects consumed their usual diet to allow all of the PSH to be excreted. For the 7 days (d 34-40 of the study) of the basal phase, the same controlled, low fiber diet and nonabsorbable marker, but no PSH supplement, were consumed. This experimental design was based on results of a preliminary study. It was observed in the preliminary study that 7-10 d of ingesting the supplement were necessary to achieve a high level of PSH excretion. Preliminary observations also

25

30

35

revealed that it took 7-10 d from ingestion of the last test dose for all of the PSH to be excreted. Therefore, the PSH period for data collection followed the 12 d of PSH consumption that constituted the screening phase and 5 a crossover experimental design was not employed.

10 Stools and qualitative bowel response, food intake and activity data were collected daily during both weeks of controlled diet. This study was approved by the College of Agricultural and Life Sciences Human Subjects Committee, University of Wisconsin-Madison.

15 **Subjects.** Twenty-one of the 33 individuals who responded to local advertisements were enrolled in the screening phase. Criteria for exclusion from the screening phase were: lactose intolerance, non-omnivorous diet and unwillingness to follow a specified diet. Subject compliance, reliability, availability and attitude were evaluated during the screening phase, and 15 subjects (8 males and 7 females) were selected to participate in the study. Fourteen 20 subjects completed the study. (Data from one subject who admitted to not providing all stools during the specified collection periods were deleted from the final analysis.) Subjects ranged in age from 18-30 yr (mean \pm SE, 24 \pm 1 yr) and were of normal body weight for height (body mass 25 index, 24.2 \pm 0.9).

30 **Diet.** All subjects consumed fixed diets during the PSH-supplemented and basal phases consisting of foods provided to them as part of the study. Breakfast was consumed at home and consisted of Rice Krispies® cereal, skim milk, orange juice, white bread, margarine or butter, and jelly. Lunch consisted of a sandwich, fresh fruit, and milk. The evening meal consisted of a meat, a starch source, salad and dessert. Subjects consumed 35 prescribed amounts of the menu foods. The amounts of bread and milk varied with daily energy needs. Limited

- 25 -

amounts of fiber-free snacks and alcohol also were permitted. Coffee was allowed *ad libitum*. Subjects were supervised at the evening meal. Consumption of the supplement and nonabsorbable marker was verified daily 5 when empty packets were exchanged for the next day's allotment of supplement.

Data and Sample Collection. All stools excreted during the PSH and basal phases of the study were individually collected, refrigerated promptly, and 10 weighed and frozen within 8 h of collection. Subjects evaluated each stool using a 9 point rating scale consisting of: -4 disagree the most, -3 disagree extremely, -2 disagree very much, -1 disagree, 0 neutral, +1 agree, +2 agree very much, +3 agree extremely, and +4 agree the most possible. 15

Subjects completed daily food intake records to verify consumption of the items in the controlled diet. Physical activity forms were completed daily to identify any major changes in daily routine. Daily routines for 20 each participant remained consistent throughout the two weeks of sample collection.

Analyses. Stools excreted during d 4-8 of each controlled diet period (d 16-20 and d 37-41 of the study) were thawed, pooled for each subject by hand mixing and 25 re-frozen until use or processed as outlined below. Duplicate aliquots (3 g) were dried (16 h, 70°C) to determine moisture content (Marlett et al., *JNCI* 76: 1065-1070, 1986). Stool chromium content was determined using a modification (Hosig et al., *Cereal Chem.* 73: 392- 30 398, 1996) of the method of Guncaga et al. (*Clin. Chim. Acta* 47: 77-81, 1974). Portions of pooled stool were lyophilized for duplicate (25 mg) determinations of neutral and amino sugar content by gas chromatography as the alditol acetate derivatives after acid hydrolysis and 35 reduction (Kraus et al., *J. Chromatog.* 513: 71-81, 1990;

- 26 -

Monsma et al., *Appl. Environ. Microbiol.* **58**: 3330-3336, 1992) and for uronic acid analysis using a colorimetric assay (Blumenkranz et al., *Anal. Biochem.* **54**: 484-489, 1973). An aqueous extract of thawed, pooled stool was 5 used to determine the relative viscosity of stool (Ostwald dropping pipet viscometer, Cat. No. 13-695, Fisher Scientific, Pittsburgh, PA). Aqueous fractions were obtained by vortexing aliquots (2 g) with water (10 ml), centrifuging (30,000xg, 30 min, 4°C), and recovering 10 and re-centrifuging the supernatant.

The fractionation procedure to isolate the component responsible for the gel characteristic was applied to thawed aliquots of pooled stool from each subject from both diet periods. Aliquots (25 g) were 15 de-lipidated and subjected to base (0.18 N KOH) containing sodium borohydride (0.026 M) to minimize base-catalyzed depolymerization. The alkali soluble fraction was acidified to pH 4.5 with glacial acetic acid and a precipitate (Fraction 1) recovered by addition to 20 ethanol. Fraction 1 was suspended in water, heated to boiling, centrifuged and the supernatant reduced in volume by roto-evaporation. The concentrate was added to ethanol to give a final alcohol concentration of 70%. The fibrous mass which formed by addition of the 25 concentrated supernatant to ethanol was washed with ethanol, ether and vacuum dried. This material represented the gelatinous component of PSH stools (Fraction 2). No precipitate formed at this point during the analysis of the control stool samples. Neutral and 30 amino sugar contents and uronic acid concentrations were determined on aliquots of this material, as outlined above.

Macronutrient and energy contents of the 35 planned menus and of the daily intakes of each subject were calculated using nutrient composition tables.

Dietary fiber intakes were calculated from food intake records using a detailed database of the fiber content and composition for US foods. The sums of the daily intakes on d 2-6 of each controlled diet period (d 14-18 and d 35-39) were used as the intake of the neutral sugars (glucose, arabinose, xylose, mannose, galactose) and the uronic acids for the determination of apparent digestibility of fiber-derived sugars. The sugar composition of the PSH supplement also was measured by gas chromatography after acid hydrolysis, as outlined above. Fecal excretion of sugars was adjusted to reflect excretion of 5 d of intake using the content of chromium in the pooled stool (Chen et al., Am. J. Clin. Nutr. 68: 711-719, 1998). Apparent digestibilities of fiber-derived sugars were calculated as the difference between intake and excretion, and expressed as a percentage of intake (Chen et al., 1998, *supra*).

Statistical Analyses. Data are reported as the mean \pm SE. Data collected during the basal and psyllium-supplemented phases of the study were compared by one-way analysis of variance using SAS computer program software release 6.12. Significant differences were identified by the least significant difference means separation test.

25

Results:

The first fraction (Fraction 1) recovered by the gel isolation scheme was extracted from both basal and PSH-containing stools; this alkali-soluble fraction from PSH stools was significantly larger ($P<0.0005$) than that extracted from basal excreta. An ethanol-precipitable fraction (Fraction 2) that was gelatinous was extracted by hot aqueous treatment of the alkali soluble fraction from stool of subjects consuming the psyllium supplement. No gelatinous fraction was

extracted from stool collected during the basal, low fiber phase of the study.

The major component in Fraction 2 isolated from stool of the 14 subjects consuming PSH was a polysaccharide that contained 763 ± 18 mg of sugar/g of Fraction 2, most of which was xylose (64 ± 1 %) and arabinose (27 ± 0 %); the remainder of the sugars was (%): 2 glucose, 3 galactose, and 3 other sugars (fucose, ribose, mannose, myoinositol, muramic acid, glucosamine and galactosamine). The xylose and arabinose in this gel fraction accounted for 53.9 ± 2.1 and 31.1 ± 1.4 % of the respective sugar in feces.

Since no fraction was obtained from basal excreta comparable to the gel isolated from the PSH-containing stool, aqueous extracts of stool were prepared for a comparison of the relative viscosity of stool from the two study periods. The apparent viscosity of the aqueous extract of the PSH-containing stool was significantly greater ($p < 0.01$) than that of the basal, low fiber excreta, 238 ± 38 vs 128 ± 7 sec.

The PSH supplement increased mean daily wet output from 117 ± 7 to 188 ± 13 g/d ($P < 0.0001$), mean daily dry output from 29 ± 2 to 37 ± 2 g/d ($P < 0.0001$), and stool moisture from 74.4 ± 0.9 % to 80.2 ± 1.0 % ($P < 0.05$). Other measures of large bowel function also were significantly different. The mean wet weight of each stool increased from 121 ± 6 to 173 ± 14 g ($P < 0.005$), mean dry weight of each stool from 30 ± 2 to 34 ± 3 g ($P < 0.0001$), and defecation frequency from 1.0 ± 0.1 to 1.1 ± 0.1 ($P < 0.05$) when PSH supplement was consumed.

The mixed food controlled diet contained a mixture of fiber-derived sugars and the apparent digestibility of the fiber-derived total neutral sugars was 67 ± 4 % during the low fiber basal period. The

- 29 -

composition of the PSH preparation was (mg/g) : 21 rhamnose, 127 arabinose, 325 xylose, 8 mannose, 25 galactose, 44 glucose, 35 uronic acids, 19 ash, 18 crude protein and 346 coating. Apparent digestibilities of the 5 fiber-derived xylose and arabinose decreased ($P<0.001$), while uronic acid digestibility increased ($P<0.03$) when the supplement was ingested. Subtracting xylose and arabinose excreted during the basal diet period from the amounts of these sugars in the PSH-containing stool 10 provides a means of estimating the apparent digestibility of the two major sugars in PSH. The apparent digestibilities of the xylose, arabinose and total neutral sugars provided by PSH were 59 ± 5 , 28 ± 7 and $54 \pm 9\%$, respectively and were variable.

15 The test dose of PSH was well tolerated by all subjects. Compared to large bowel function during the basal period, PSH resulted in gentler bowel movements, softer stools that were easier to pass, greater ease of wiping, a feeling a complete relief, and increased bulk.

20 The PSH supplement had no effect on abdominal cramping, an urge to defecate, or experience a repeat bowel evacuation, nor did it cause diarrhea, although subjects perceived more flatulence and bloating. Although soft and formed, most stools were visibly gelatinous and would 25 vibrate when agitated. The macronutrient and dietary fiber contents provided by the foods in the diet did not change when the PSH supplement was consumed.

Thus, in contrast to other viscous fibers that are completely fermented in the colon, a component of 30 psyllium is not fermented. The results of the foregoing study indicate that, by functioning as an emollient and a lubricant, the unfermented gel isolated from PSH-containing stool represents a new mechanism of laxation for a dietary fiber.

EXAMPLE 7

Demonstration of Poor Fermentation of the
Gel-Forming Fraction of Psyllium Seed Husks In Vitro

5 *In vitro* fermentations of psyllium seed husks and its fractions were conducted in 40 ml fermentation flasks. Carbohydrate for fermentation was 200 mg, of which 31 mg was from veal infusion broth and 45 mg was from yeast; the remainder was from Fractions A, B and C 10 of psyllium seed husks. Fractions were isolated from ground psyllium seed husks as described above. The "3 fractions combined" sample consisted of the three fractions isolated from the psyllium husk re-combined in proportion to their original concentration in the husks. 15 Inoculum consisted of cecal contents harvested from rats fed purified diets containing 5% (by weight) psyllium seed husks. Experimental protocols and analyses were according to the methods described by Monsma & Marlett (J. Nutr. 126: 554-563, 1996). Results generated by this 20 methodology have been shown to be similar to results obtained by *in vivo* fermentation in humans. (Monsma et al., J. Nutr. 130: 585-593, 2000). All fermentations were prepared and conducted in an anaerobic chamber, and were performed in duplicate.

25 Table 5 shows the results of the *in vitro* fermentation. Carbohydrate in the fermentation flasks consisted of eleven neutral and amino sugars, along with uronic acids; these were provided by the test substrate veal infusion broth and yeast (see Table 5 footnotes "a" 30 and "b"). The apparent fermentation of the sugars from these sources is summarized such that the fermentation of the test fractions is highlighted. As can be seen from the table, Fraction C was almost completely fermented by 24 hours. By contrast, Fraction B was only partially 35 fermented (approx. 25-35%), and its fermentation appeared to have ceased by 48 hours. Fraction A was poorly fermented, and its fermentation was limited to the first 12 hours of the fermentation period.

Table 5. Disappearance of neutral sugars during *in vitro* fermentation of psyllium seed husk and its fractions

	Polysaccharide/sugar	0 h		12 h	24 h	48 h	72 h
		buffer ^a	fract ^b --mg/flask--				
5	Psyllium husk						
10	rhamnose	0.6	4.5	21	22	21	22
	arabinose	0.9	28.1	84	84	76	82
	xylose	2.6	69.3	68	46	39	40
	galactose	4.3	5.4	61	67	61	66
	others ^c	65.5	9.2	23	22	19	21
15	3 fractions combined						
20	rha	0.6	4.4	19	22	22	22
	ara	0.9	29.9	85	81	76	80
	xyl	2.6	70.0	73	47	44	44
	gal	4.3	5.7	56	61	58	60
	others	65.5	8.6	24	22	21	20
25	Fraction C						
30	rha	0.6	21.4	16	6	6	6
	ara	0.9	11.6	47	19	18	19
	xyl	2.6	57.3	53	11	9	8
	uronic acids	3.1	30.5	22	tr	tr	tr
	others	69.9	3.6	14	12	8	9
35	Fraction B						
40	rha	0.6	1.3	79	61	52	53
	ara	0.9	26.8	93	85	78	76
	xyl	2.6	91.4	84	76	65	59
	others	69.9	2.2	13	10	10	10
	Fraction A						
45	ara	0.9	49.2	100	100	100	100
	xyl	2.6	4.6	68	58	59	59
	man	15.4	16.0	56	58	65	55
	gal	4.3	19.8	91	89	94	98
	glc	35.3	29.4	53	50	54	55
50	others	17.4	0.7	27	23	19	19

a Sugars in the veal infusion broth and yeast that are part of the fermentate and contributed about 70-75 mg of carbohydrate to the fermentation.

b Sugars in the test substrate, calculated to contribute about 120-125 mg to the fermentation.

c Includes those sugars from the following list that are not specifically listed: rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose, glucose, myoinositol, glucosamine and galactosamine. Although uronic acids were included in the calculation of the amount of carbohydrate to be fermented, they have not been measured in all samples and therefore are not included in this summary, except for the Fraction C summary.

5

EXAMPLE 8
Reductive Cleavage and NMR Analysis
of Psyllium Seed Husk Fraction B

The polysaccharide of psyllium seed husk
 10 Fraction B was methylated fully, then reductively cleaved
 in the presence of triethylsilane and trimethylsilyl
 trifluoromethanesulfonate, according to standard methods.
 The products were acetylated *in situ* and analyzed by gas-
 liquid chromatography - mass spectrometry (GLC-MS) on a
 15 DB-5 column. Results are shown in Table 6.

Table 6. Summary of Reductive Cleavage Analysis

	Peak	Residue	Mole Percent
	1	Terminal Xylp	23.27
20	2	Terminal Araf	16.95
	3	3-Linked Araf	10.76
	4	3-Linked Xylp	4.33
25	5	Terminal Galp	1.07
	6	2,3- or 3,4-Linked Xylp	11.84 ^a
	7	2,3-Linked Araf	3.44
30	8	2,4-Linked Xylp	21.41 ^b
		3,5-Linked Araf	0.58 ^b
	9	2,5-Linked Xylf	2.68
35	10	Unidentified	
	11	3-Linked Galp	0.66 ^b
		2,3,4-Linked Xylp	3.01 ^b

40 ^a Standard methylation analysis established that this Xylp residue
 was 3,4-linked.

^b Coeluting components were separated by GLC on an RTx-200 column.

The components of a reductive cleavage process are more easily separated for identity and quantitation and provide more definitive structural information than those from a standard methylation/hydrolysis procedure.

5 The data set forth in Table 6 indicate that the polysaccharide of Fraction B is a highly branched, very complex molecule. The xylan backbone contains, in addition to single branches of xylose and arabinose, branches of an indeterminate length that may contain both
10 sugars.

The polysaccharide was also subjected to two-dimensional NMR analysis, the results of which indicated that the xylose residues are beta-linked and the arabinose residues are alpha-linked. The latter
15 assignment is supported by the action of alpha-L-arabinofuranosidase on the polysaccharide.

The present invention is not limited to the embodiments described and exemplified above, but is
20 capable of variation and modification without departure from the scope of the appended claims.

We claim:

1. A gel fraction of psyllium seed husks that survives microbial fermentation upon passage through a monogastric mammalian digestive tract, said fraction comprising xylose and arabinose in a dry weight ratio of at least about 3:1 and further comprising less than about 2% (by weight) rhamnose.
- 10 2. The gel fraction of claim 1, wherein the xylose to rhamnose dry weight ratio is greater than 50.
- 15 3. The gel fraction of claim 1, which comprises galactose, having a xylose to galactose dry weight ratio that is greater than 25.
- 20 4. The gel fraction of claim 1, which comprises uronic acids, having a xylose to uronic acids dry weight ratio that is greater than 25.
- 25 5. The gel fraction of claim 1, having a sugar composition, based on percent dry weight, of:
 - between about 0 and 3.5% rhamnose;
 - between about 15 and 20% arabinose;
 - between about 55 and 70% xylose;
 - between about 0 and 0.5% mannose;
 - between about 1 and 2% galactose
 - between about 0 and 0.5% glucose; and
 - between about 0.5 and 5% uronic acids.
- 30 6. The gel fraction of claim 1, having an apparent viscosity in formamide of at least 500 sec.
- 35 7. The gel fraction of claim 1, which is soluble in a dilute alkaline solution and which forms a

gel upon acidification of the solution to a final pH of about 4.5.

8. A pharmaceutical preparation for treatment
5 of constipation in a patient in need of such treatment,
comprising an effective dose of the gel fraction of claim
1.

9. The pharmaceutical preparation of claim 8,
10 wherein the effective dose is between about 2 and about 6
g, based on dry weight, of the gel.

10. A pharmaceutical preparation for treatment
of high blood cholesterol in a patient in need of such
15 treatment, comprising an effective dose of the gel
fraction of claim 1.

11. The pharmaceutical preparation of claim
10, wherein the effective dose is between about 3 and
20 about 7 g, based on dry weight, of the gel.

12. A carbohydrate fraction of psyllium seed
husks, said fraction being soluble in a dilute alkaline
solution and remaining soluble upon acidification of the
25 solution to a pH of about 4.5, said fraction comprising
xylose and arabinose in a ratio of at least about 4:1 and
further comprising at least about 12% (by weight)
rhamnose and at least about 15% (by weight) uronic acid.

30 13. A method of fractionating psyllium seed
husks to obtain a gel fraction and an additional
carbohydrate fraction, the method comprising:

35 a) mixing the husks in an aqueous alkaline
solution comprising between about 0.15 and 1.0 M hydroxyl
ions, thereby fractionating the husks into an alkali

- 36 -

soluble fraction and an alkali-insoluble fraction;
b) removing the alkali insoluble fraction;
c) acidifying the alkali soluble fraction
to a pH of between about 3 and 6, thereby obtaining a gel
5 fraction, and an acidified solution comprising the
additional carbohydrate fraction; and
d) separating the gel fraction from the
solution containing additional carbohydrate fraction.

10 14. The method of claim 13, wherein the husks
are mixed in an aqueous alkaline solution comprising
between about 0.15 and 0.3 M hydroxyl ions.

15 15. The method of claim 13, wherein the alkali
soluble fraction is acidified to a pH of about 4.5.

16. The method of claim 13, which further
comprises washing the gel fraction with an aqueous
solution.

20 17. The method of claim 16, which further
comprises desiccating the washed gel fraction.

25 18. A gel fraction from psyllium seed husks,
produced by the method of claim 13.

19. A pharmaceutical preparation for treatment
of constipation in a patient in need of such treatment,
comprising an effective dose of the gel fraction of claim
30 18.

20. The pharmaceutical preparation of claim
19, wherein the effective dose is between about 2 and
about 6 g, based on dry weight, of the gel.

21. A pharmaceutical preparation for treatment of high blood cholesterol in a patient in need of such treatment, comprising an effective dose of the gel fraction of claim 18.

5

22. The pharmaceutical preparation of claim 21, wherein the effective dose is between about 3 and about 7 g, based on dry weight, of the gel.

10

23. A carbohydrate fraction from psyllium seed husks, produced by the method of claim 13.

15

24. A method of treating constipation in a patient in need of such treatment, which comprises administering to the patient the pharmaceutical preparation of claim 8, in an amount and for a time effective to relieve the constipation.

20

25. A method of treating constipation in a patient in need of such treatment, which comprises administering to the patient the pharmaceutical preparation of claim 18, in an amount and for a time effective to relieve the constipation.

25

26. A method of lowering serum cholesterol in a patient in need of such treatment, which comprises administering to the patient the pharmaceutical preparation of claim 8, in an amount and for a time effective to lower the patient's serum cholesterol.

30

27. A method of lowering serum cholesterol in a patient in need of such treatment, which comprises administering to the patient the pharmaceutical preparation of claim 18, in an amount and for a time effective to lower the patient's serum cholesterol.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/15693

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 31/715, 35/78

US CL :514/54; 424/195.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/54; 424/195.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

psyllium, gel, polysaccharide, alkali, xylose, arabinose, rhamnose, galactose, galacturonic acid, glucose, mannose

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LAIDLAW et al. Studies of Seed Mucilages. Part V. Examination of a Polysaccharide extracted from the Seeds of Plantago ovata Forsk by Hot Water. Journal of the Chemical Society. 1950, pages 528-534, especially page 529.	1-4, 6-11, 18-23 -----
Y		24-27
X	LAIDLAW et al. Studies on Seed Mucilages. Part III. Examination of a Polysaccharide extracted from the Seeds of Plantago ovata Forsk. Journal of the Chemical Society. 1949, pages 1600-1608, especially page 1600.	12
X	KENNEDY et al. Structural Data for the Carbohydrate of Ispaghula Husk ex Plantago ovata Forsk. Carbohydrate Research. 1979, Vol. 75, pages 265-274, especially pages 266, 271 and 273.	13-23 -----
Y		24-27

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*'A'	document defining the general state of the art which is not considered to be of particular relevance	
*'B'	earlier document published on or after the international filing date	"X"
*'L'	document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"
*'O'	document referring to an oral disclosure, use, exhibition or other means	"Z"
*'P'	document published prior to the international filing date but later than the priority date claimed	document member of the same patent family

Date of the actual completion of the international search

02 AUGUST 2000

Date of mailing of the international search report

06 SEP 2000

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

FRANCISCO C. PRATS

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/15693

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SANDHU et al. The Gel Nature and Structure of the Carbohydrate of Ispaghula Husk ex Plantago ovata Forsk. Carbohydrate Research. 1981, Vol. 93, pages 247-259, especially page 253,	13-23
Y	US 5,234,916 A (HORD) 10 August 1993, see whole document.	24-27
Y	US 5,248,502 A (NDIFE) 28 September 1993.	24-27
A		1-27